

Prediction of Heparin binding sites on Human Serum Albumin, Matrix Metalloproteinase-2 and DNA Topoisomerase1

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ABSTRACT:

Heparin binds a wide range of proteins of different structure as well as functions and play crucial roles in a number of biological processes. Human seminal plasma consists of many heparin binding proteins (HBPs). HBPs play crucial role in modulation of capacitation through heparin and have been correlated with fertility in many species. Very little scientific information is available about the binding modes of heparin and HBPs. There is not any well defined binding space, characterized by some consensus sequence over protein except that binding region always do consist of basic residues. Thus, we have to study each heparin-HBPs complex individually to gather information about their interactions. Here, we are reporting the results of docking studies to predict the heparin binding sites of three human seminal fluid HBPs (human serum albumin, matrix metalloproteinase-2 and DNA topoisomerase 1) using program Dock6. Our docking results show that Arg117, Arg186, Lys519 of human serum albumin, Lys531, Tyr540, Glu549, Tyr552, Tyr591, Lys596 of matrix metalloproteinase and Lys439, Lys443, Arg488, Thr585, Lys587, Thr591 of DNA topoisomerase1 make hydrogen bond contacts with heparin. The positively charged residues Arginine and Lysine play important role in their interactions. Our study may add up to better understanding of protein-heparin binding modes.

Keywords: Heparin binding proteins, Molecular docking, Dock6, Protein structure

INTRODUCTION

Heparin is a polysaccharide which is highly sulfated and belongs to the family of glycosaminoglycans [1]. It plays important roles in various biological activities due to its interaction with a wide range of proteins [2]. Heparin plays a key role in enhancement of sperm capacitation, motility and the acrosome reaction [3, 4]. It also helps in initiation of sperm nuclei decondensation in more than seven animal species by inducing changes in chromatin [5]. Heparin- protein interactions show varying physiological functions and the structural basis of these interactions is not very clear. The binding of heparin to proteins is considered non-specific in nature due to the fact that heparin is highly anionic. The very little known about these interactions is that the positively charged residues of proteins and negatively charged sulfates of heparins play key roles in these interactions. The other factors which contribute to the stability of heparin-protein complexes are non-electrostatic interactions such as hydrogen bonding and hydrophobic interactions [6]. Molecular modeling studies of heparin-protein interactions have revealed some consensus sequences for heparin binding like XBBXB and XBBBXXBX [7], BBXB [8, 9], XBBX, XBBB and BXXB [10], where B is a basic amino acid and X can be any amino acid. There are not sufficient evidences to show the common features which can efficiently be used for predicting heparin binding sites. But the studies so far exposed one common feature that all heparin binding sites are characterized by positively charged residues [11]. Secondly, HBPs fold into structures that differ widely. Thus, it is not possible to predict heparin

binding sites through sequence analysis and we have to study each heparin-protein complex individually to find their binding modes [12].

The presence of spermatozoa at the site of fertilization and its capacitation are essentials for fertilization to occur [13]. The acrosome reaction is next critical step where the spermatozoon outer acrosomal and overlying plasma membranes fuse and rest of the parts are released from the egg-sperm complex [14]. Capacitation, a prerequisite for acrosome reaction is enhanced by glycosaminoglycan (GAGs) [15-17]. Among the numerous GAGs studied, heparin is the most potent enhancer of capacitation in bovine and rabbit [17]. Human seminal plasma contains several proteins that bind heparin and related glycosaminoglycans [18]. Heparin binding proteins (HBPs) from bovine seminal plasma have been previously studied and the results documented the role of HBPs in modulation of capacitation. HBPs bind to spermatozoa and then modulate the capacitation through heparin. Thus, these are correlated with the fertility issues [19, 20]. Our group has previously purified seven heparin binding proteins from human seminal plasma. We have also done proteomic studies of HBPs of human seminal plasma and identified 40 HBPs [21]. Human serum albumin, DNA topoisomerase1 and Matrix metalloproteinase-2 were identified as heparin binding proteins in this study.

The molecular docking techniques can efficiently be employed to predict the low energy binding modes of small ligands to macromolecules. Docking refers to the

in-silico studies of binding modes of two molecules. It processes biomolecules to predict the way in which they interact and fit together. During the past decade there has been a great enhancement in the computational machine powers in the terms of memory and processing speed and the structural databases are flooded with the new known structures. Due to enhanced processing speed of computers and more molecular structural information available in the databases, it is now possible to simulate and analyze docking predictions in far easier way than past times. There are various docking tools available which efficiently solve this purpose.

METHODOLOGY

3-D crystal structures of HBPs [Human serum albumin (1AO6), Matrix metalloproteinase-2 (1RTG), DNA topoisomerase1 (1EJ9)] were retrieved from Protein Data Bank [22]. 3-D crystal structure of fibroblast growth factor complexed with heparin (PDB ID: 1BFB) was edited to retrieve heparin crystal structure. The PDB structures are only raw data for docking and cannot be used directly for docking analysis. These structures are needed to be edited before being used as input for docking program. These PDB files were processed using UCSF Chimera tools [23] for adding hydrogen, charge and dock prep. Various MOL2 and PDB format files were prepared to be used as docking input.

Molecular docking of the receptor (protein) and ligand (heparin) were done with the default options using the DOCK program Version 6.2 [24] running on Fedora8. This program addresses automatically the flexible docking of the ligands into a known protein structure. The main steps of docking method include site characterization, grid computation and docking calculations. The molecular surface of receptor proteins was generated using program *dms*. Within the DOCK suite of programs, *sphgen* identifies the active site, and other sites of interest, and generates the sphere centers that fill the site. *Grid* generates the energy scoring grids and *DOCK* matches spheres with ligand atoms and uses scoring grids to evaluate ligand orientations. Chimera was used as an accessory tool for visualizing and analyzing docking outputs.

RESULTS AND DISCUSSION

Our main objective for this study was to predict heparin binding sites of proteins and use this information to find if these sites share some common features. The output files of dock program were analyzed on Chimera to find which residues of proteins

are important for heparin binding (Table 1). We also selected the residues which make hydrogen bond contacts with heparin molecule.

Human serum albumin-heparin complex (Figure 1) shows that heparin binding site consists of Arg114, Leu115, Val116, Arg117, Arg145, Arg186, Lys190 and Lys519 (residue numbers are from original PDB files). Out of these residues Arg117, Arg186 and Lys519 make hydrogen bond contacts with heparin. Sequence analysis reveals that some of the interacting residues make clusters of previously defined consensus sequences such that Arg114-Arg117 (BXXB) and Ala143-Pro147 (XBBBX) as suggested by Fromm et al. [10].

In matrix metalloproteinase 2-heparin complex, Gln528, Glu 529, Lys531, Tyr540, Ile542, Glu549, Tyr552, Leu559, Tyr591, Glu593, Lys595, Lys596 and Lys597 are residues which build heparin binding site (Figure 2). Residues Lys531, Tyr540, Glu549, Tyr552, Tyr591 and Lys596 make hydrogen bond contacts. Matrix metalloproteinase-2 sequence consists of consensus sequence XBBBX at position Val594-Met598 which is vital for heparin binding.

Residues Lys439, Asp440, Lys443, Arg488, Lys532, Thr585, Lys587, Thr591, Asn722 and Phe723 of DNA topoisomerase1 construct heparin binding site (Figure 3) and Lys439, Lys443, Arg488, Thr585, Lys587 and Thr591 bind to heparin. DNA topoisomerase1 sequence consists of two BXXB clusters (Lys436-Lys439 and Lys587-Arg590). These clusters are hot spots of heparin binding site.

When we analysed heparin binding site residues in DNA topoisomerase1, we found that the site is characterized by clusters of a new sequence BXXXB. This sequence pattern is located at positions Lys439-Lys443, Lys484-Arg488 and Lys532-Arg536. All three clusters are required for the formation of heparin binding site and protein folding has brought them closer in 3-D structure. Predicted heparin binding site in human serum albumin also consists of one such sequence pattern at position Arg186-Lys190. All the results clearly indicate that positively charged residues Lysine and Arginine are very essential for interaction and binding of heparin to HBPs. These positively charged residues either make clusters in the sequence or the protein folding has brought them closer in 3-D structure to interact with heparin. Thus, these predictions are in approval with other studies done to locate heparin binding sites and may add up to better understanding of protein-heparin binding modes.

Table 1: list of residues of different HBPs which are critical for heparin interaction

HBPs	Heparin binding site residues	Residues making H-bond contacts
Human serum albumin	Arg114, Leu115, Val116, Arg117, Arg 145, Arg186, Lys190, Lys519	Arg117, Arg186, Lys519
Matrix metalloproteinase-2	Gln528, Glu 529, Lys531, Tyr540, Ile542, Glu549, Tyr552, Leu559, Tyr591, Glu593, Lys595, Lys596, Lys597	Lys531, Tyr540, Glu549, Tyr552, Tyr591, Lys596
DNA topoisomerase1	Lys439, Asp440, Lys443, Arg488, Lys532, Thr585, Lys587, Thr591, Asn722, Phe723	Lys439, Lys443, Arg488, Thr585, Lys587, Thr591

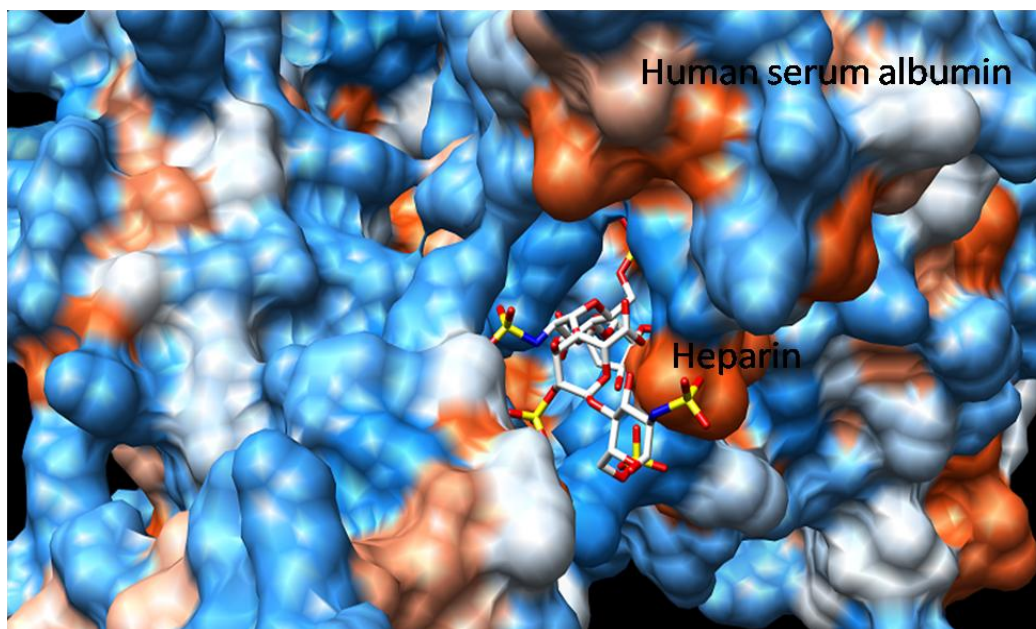


Figure 1: (A) Heparin (stick model) docked on surface of human serum albumin.

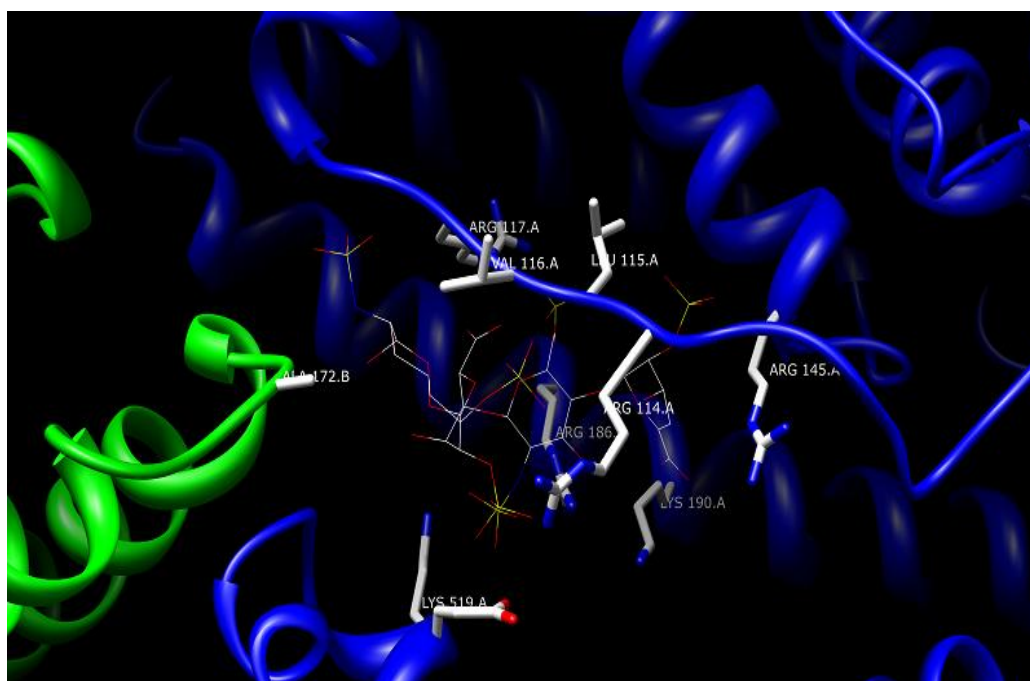


Figure 1: (B) Residues of albumin (ribbon model) interacting with heparin (wire model)

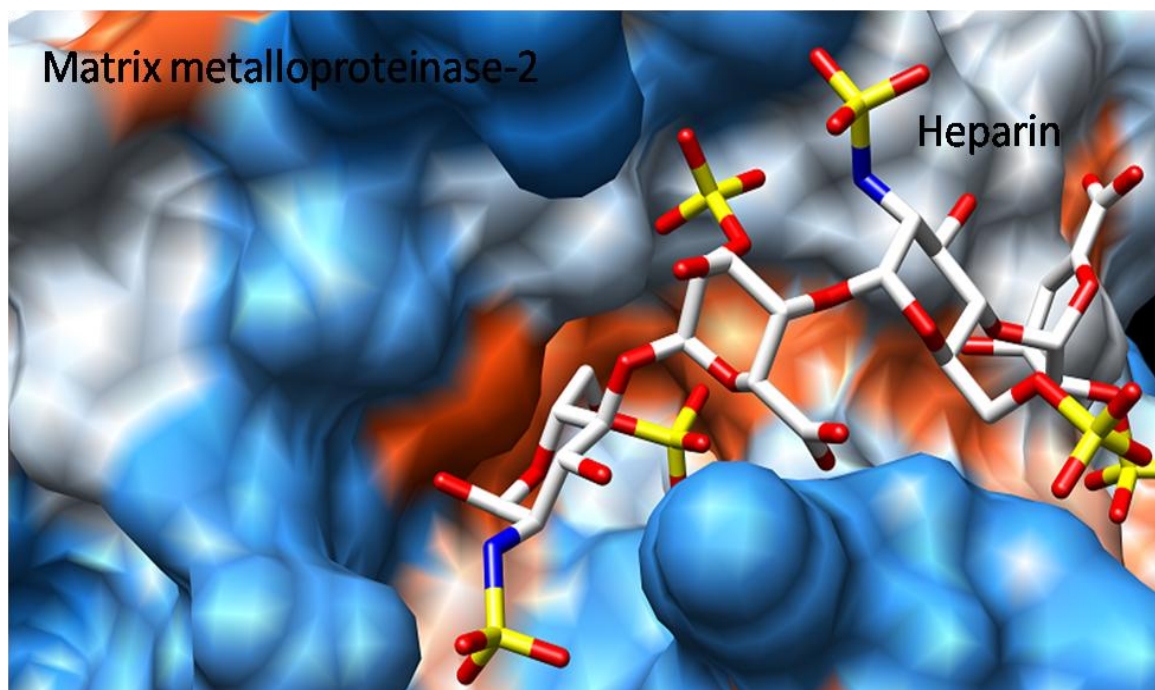


Figure 2: (A) Heparin (stick model) docked on surface of matrix metalloproteinase-2.

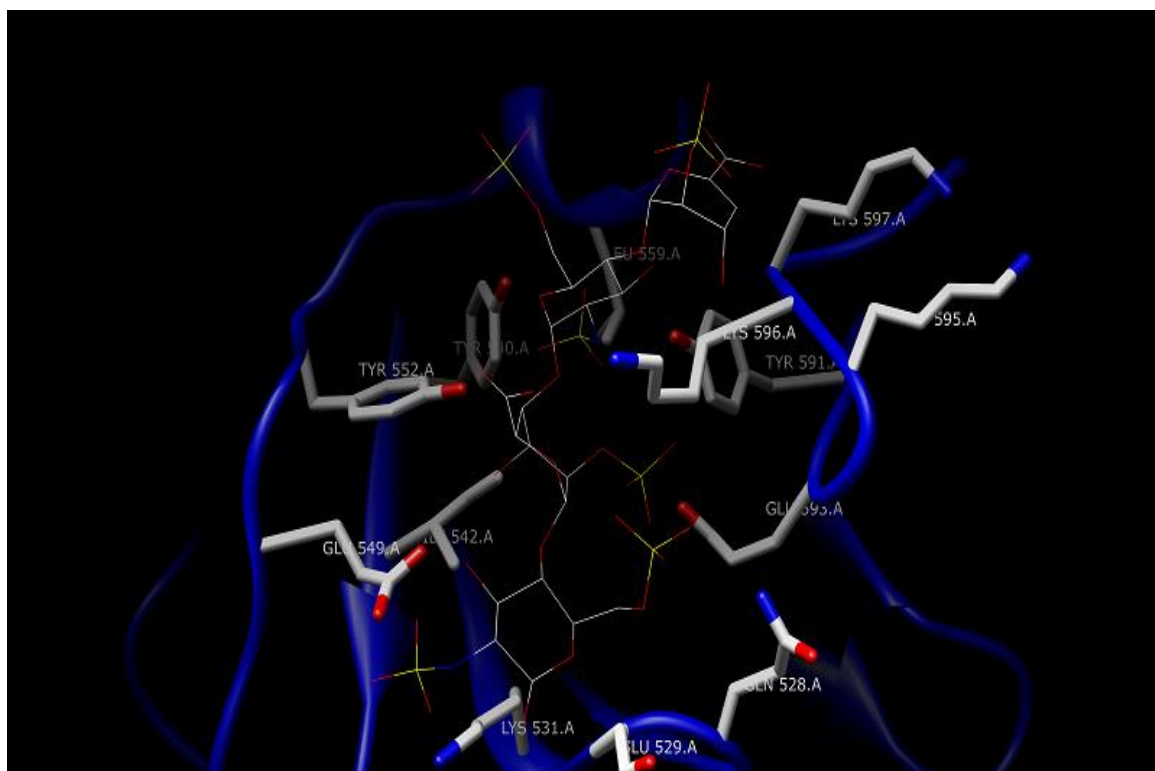


Figure 2: (B) Residues of matrix metalloproteinase-2 (ribbon model) interacting with heparin (wire model)

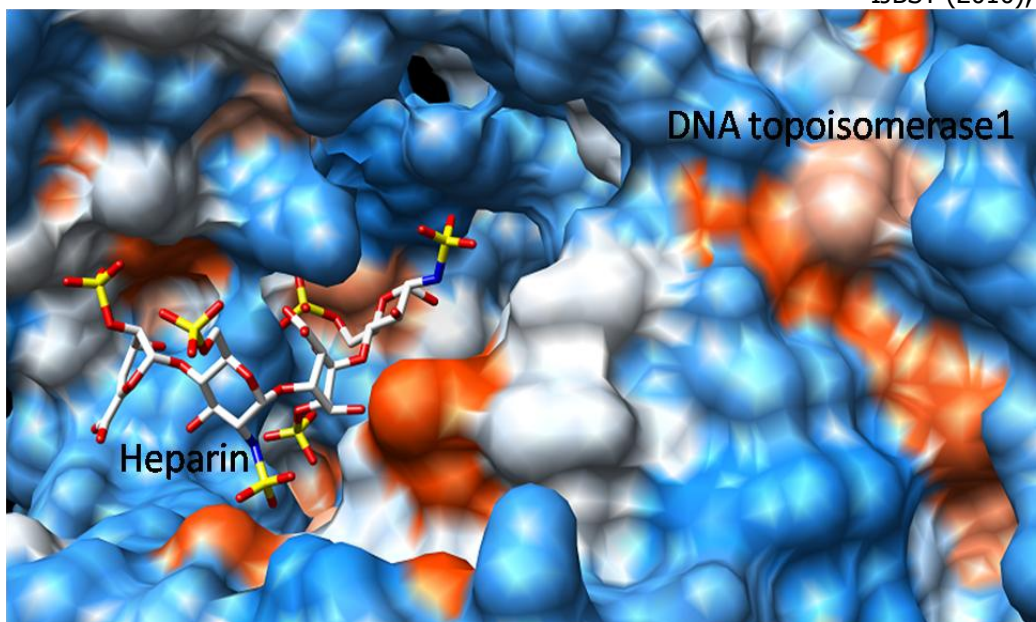


Figure 3: (A) Heparin (stick model) docked on surface of DNA topoisomerase1.

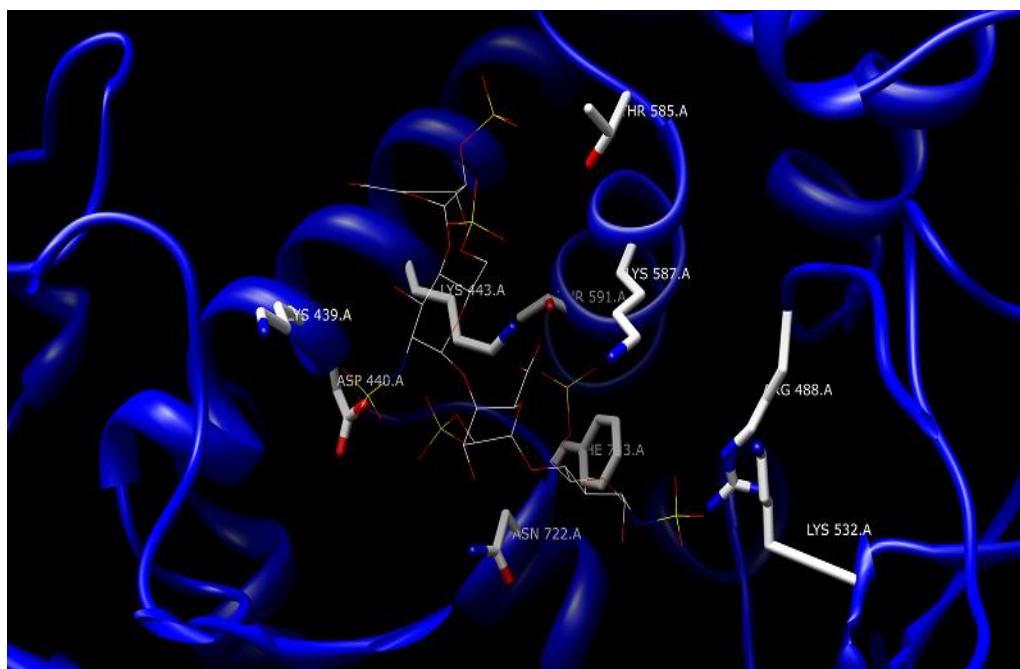


Figure 3: (B) Residues of DNA topoisomerase1 (ribbon model) interacting with heparin (wire model)

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